The Partial Enzymatic Hydrolysis of Yeast Nucleic Acid[a]

DISSERTATION

Submitted to the Board of University Studies of the Johns Hopkins University, in Conformity with the Requirements for the Degree of Doctor of Philosophy

BY

ANNABELLA E. RICHARDS

1914

[a] Published in part in the following articles:

Walter Jones and A. E. Richards (1914), The Partial Enzymatic Hydrolysis of Yeast Nucleic Acid. J. Biol. Chem., 17, 71.

Walter Jones and A. E. Richards (1915), Simpler Nucleotides from Yeast Nucleic Acid. J. Biol. Chem., 20, 25.

781
65R
UNCLASSED
ssertation
STANFORD UNIVERSITY LIBRARIES
WITHDRAWN

The Partial Enzymatic Hydrolysis of Yeast Nucleic Acid[a]

DISSERTATION

Submitted to the Board of University Studies of the Johns Hopkins University, in Conformity with the Requirements for the Degree of Doctor of Philosophy

BY

ANNABELLA E. RICHARDS

1914

[a] Published in part in the following articles:

Walter Jones and A. E. Richards (1914), The Partial Enzymatic Hydrolysis of Yeast Nucleic Acid. J. Biol. Chem., 17, 71.

Walter Jones and A. E. Richards (1915), Simpler Nucleotides from Yeast Nucleic Acid. J. Biol. Chem., 20, 25.

ACKNOWLEDGMENT

This research was carried out in the Laboratory of Physiological Chemistry, under the direction of Professor Walter Jones, to whom the writer desires to express her appreciation of the advantages which have been enjoyed.

HISTORICAL

In the year 1874 the distinguished physiologist, Friedrich Miescher,[1] made an extensive study of the habits of the Rhine salmon in fresh water. By comparative observations of animals captured at various points of the river, between its mouth and the spawning beds at Frankfurt, he was able to prove an older suspicion that these fish never feed while ascending the river; for with very rare and easily explained exceptions the alimentary canal was found free from food detritus and the digestive fluids were inactive. As the muscle tissue had greatly decreased during this journey, while both the male and female organs of reproduction had grown enormously, Miescher drew the obvious conclusion that eggs and spermatozoa had been formed at the expense of muscle protein; so that the principle chemical constituents of these tissues could reasonably be ascribed to a protein precursor.

During the spawning season the spermatic fluid (lachsmilch) can be obtained from these animals in any desired amount and when expressed from the "vas deferens" of a living fish consists of little more than spermatozoa suspended in a dilute salt solution. Upon microscopic examination the spermatozoa were seen to consist of head, tail and connecting part "Mittestuch," it being especially characteristic that the two latter parts together make up a mass that is insignificant in comparison with the mass of the head, while the tails are threads of extreme fineness, which may be easily dissolved with acetic acid, leaving the heads as insoluble granular masses.

---

[1] F. Miescher (1874), Die Spermatozoen einiger Wirbelthiere. Verhandl. d. Naturforsch. Ges. in Basel, 6, 138. Die histochemischen und physiologischen Arbeiten von Friedrich Miescher, Leipzig, 1897, 55.

From comparative histological studies of the growing testicle it becomes apparent that the spermatozoa head is to be regarded as a cell nucleus, so that Miescher gained possession of material in great quantity, which was admirably adapted to a chemical examination of the cell nucleus. He found the spermatozoa heads free from protein, and *made up almost entirely of a single chemical individual, a salt of an organic acid, rich in phosphorus.* This substance was the first known member of a large group of acids, now called *Nucleic Acids,* some member of which is present in the nucleus of every animal and plant cell. While the biological distribution of the nucleic acids is very wide, nearly all the chemical information which we possess of the members of this group was originally acquired from researches executed with two substances, one of which is prepared from yeast (Yeast Nucleic Acid) and the other from the thymus gland (Thymus Nucleic Acid). Both of these substances were submitted to chemical examination for the first time by Kossel, whose widely known and highly esteemed investigations culminated in the discovery that thymus nucleic acid is composed of six groups, so that when it is submitted to hydrolysis with mineral acids it produces the six substances, guanine, adenine, cytosine, thymine, phosphoric acid and hexose.[2]

Kossel also showed that while yeast nucleic acid contains groups very similar to those of thymus nucleic acid, it, nevertheless, gives uracil instead of thymine[3] and pentose instead of

---

[2] A. Kossel and A. Neuman (1894), Darstellung und Spaltungsproducte der Nucleinsäure. Ber. d. d. chem. Ges., 27, 2215.

[3] A. Ascoli (1900), Uber ein Neues Spaltungsprodukt des Hefe Nucleins. Zeits. f. physiol. Chem., 31,161.

hexose.[4] A comparison of the two nucleic acids in this respect is made in the following table.

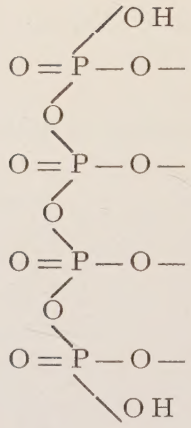
HYDROLYTIC PRODUCTS

Thymus Nucleic Acid (Animal Nucleic Acid)	Yeast Nucleic acid (Plant Nucleic Acid)
Phosphoric acid.	Phosphoric acid.
Guanine.	Guanine.
Adenine.	Adenine.
Cytosine.	Cytosine.
Thymine.	Uracil.
Hexose (i e., levulinic acid and formic acid).	Pentose.

The facts embraced in this table are among the most firmly established conclusions of physiological chemistry and constitute the source from which all the more recent discussions of the subject have proceeded. They serve to characterize the two types into which nucleic acids naturally fall, for all substances of this class have been found to yield one or the other of these sets of hydrolytic products. To solve the constitution of nucleic acids it therefore remained only to find the order in which the six groups are arranged. In this direction also Kossel made the first advance,[5] when he obtained polyphosphoric acid from yeast nucleic acid and thus furnished a strong indication that the nucleic acid has at the base of its structure some such complex as that represented by the formula:

[4] A. Kossel (1893), Uber die Nucleinsäure. Archiv. f. (Anat. u.) Physiol., 157.

[5] A. Kossel (1900), Zur Abwehr. Zeitchr. f. physiol. Chem., 31,428.



At this point the subject assumed a little complication when Ivar Bang[6] introduced a compound called guanylic acid, which he obtained from the pancreas and which he believed to yield on hydrolysis phosphoric acid, guanine, pentose and *glycerine*. This complication, to which the discovery of guanylic acid gave rise, was considerably simplified when Steudel[7] found that the substance does not produce glycerine on hydrolysis. But there remained, and remains to this day the anomaly that animal glands contain a substance which is chemically related to nucleic acid, since it produces both phosphoric acid and guanine, but can scarcely be physiologically related to the nucleic acid of animal cell nuclei, since it contains a pentose group, that is to say, the carbohydrate group of plant nucleic acid.

---

[6] Ivar Bang (1898), Die Guanylsäure der Pankreasdrüse und deren spaltungsproducte. Zeits f. physiol. Chem., 26, 133.

[7] H. Steudel (1907), Ueber Guanylsäure aus der Pankreasdrüse, ibid, 53, p. 539.

While this matter was undergoing considerable speculation it was found that guanylic acid is not confined to the pancreas, but is very widely distributed in animal glands,[8] and shortly afterwards Levene and Jacobs[9] solved its chemical constitution. By submitting the substance to neutral hydrolysis under pressure they found that it lost phosphoric acid, giving a beautifully crystalline nucleoside called guanosine, which in turn yields pentose and guanine.

$$\begin{matrix} H_2O \\ \quad O = P - O.\ C_5H_8O_3.\ C_5H_4N_5O + H_2O = \\ HO \end{matrix}$$

$$H_3PO_4 + \underset{\text{guanosine}}{C_5H_9O_4.\ C_5H_4N_5O}$$

$$\underset{\text{guanosine}}{C_5H_9O_4.\ C_5H_4N_5O} + H_2O =$$

$$C_5H_{10}O_5 + \underset{\text{guanine}}{C_5H_5N_5O}$$

Thus guanylic acid is representative of a class of substances called *nucleotides,* i. e., compounds in which groups of phosphoric acid and nitrogenous rings are linked by carbohydrate groups.

Levene and Jacobs[10] then applied to yeast nucleic acid the method which they had successfully employed with guanylic

---

[8] R. Odenius (1900), Einige Untersuchungen über ein Nucleoproteide der Milchdrüse Malys Jahresbericht 33.

W. Jones and L. G. Rownrtee (1908), On the Guanylic acid of the Spleen. J. Biol. Chem. 4,239.

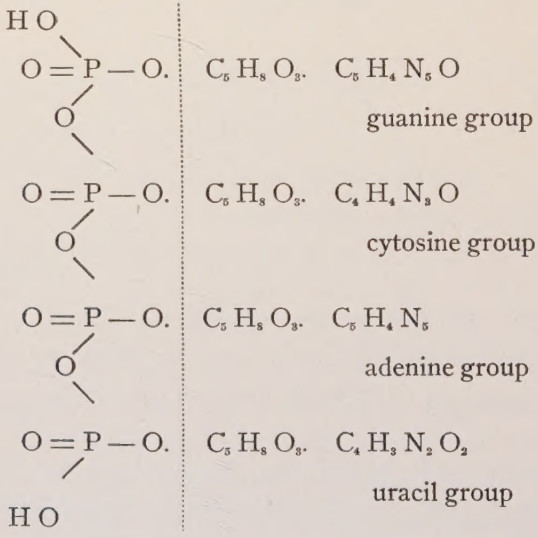
P. A. Levene and J. A. Mandel (1908), Zur Chemie der Lebernucleoproteid. Biochem. Zeits., 10,221.

[9] P. A. Levene and W. A. Jacobs (1909), Ueber Guanylsäure. Ber. d. d. Chem. Ges., 42, 2469.

[10] P. A. Levene and W. A. Jacobs (1909), Uber die Hefenucleinsäure. Ibid, 42, 2474.

P. A. Levene and W. A. Jacobs (1909), Uber die Pentose in den Nucleinsäure. Ibid, 42, 3247.

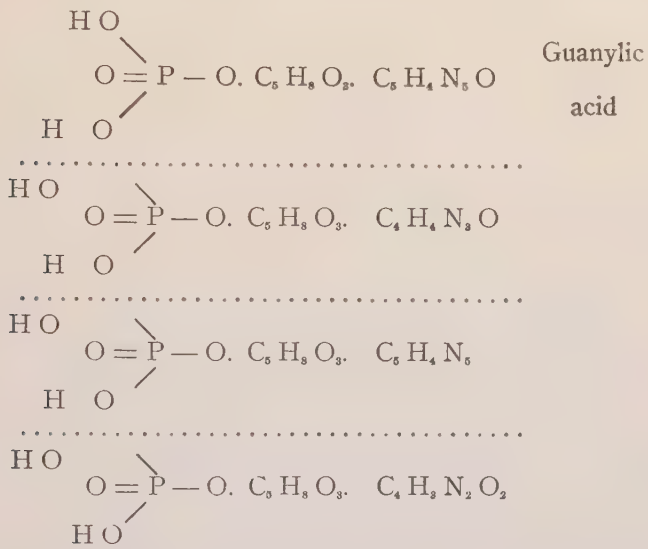
acid, and found that by neutral hydrolysis under pressure the substance loses phosphoric acid and yields *four* nucleosides; one of which is identical with the nucleoside guanosine, similarly obtained from guanylic acid. It would therefore appear that as guanylic acid is a *mono*-nucleotide, yeast nucleic acid is a tetra-nucleotide, and is composed of four mono-nucleotide groups, as is seen in the following formula:



EXPLANATION OF RESULTS.

The dotted line of the formula above indicates how the four nucleosides must of necessity be produced when nucleic acid is decomposed in such a way as to completely lose its phosphoric acid, whether the decomposition occur directly or indirectly. But if hydrolysis should occur in such a way as to bring about a disruption of the phosphoric acid groups from one another, without altering the compound in any other way,

four mono-nucleotides should be formed, one of which must be identical with guanylic acid. This is seen in the following diagram:—



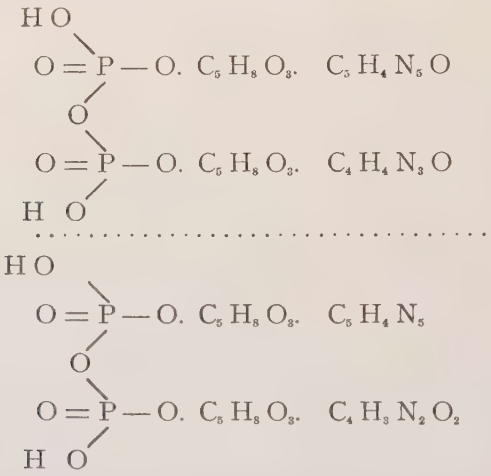
To obtain guanylic acid from yeast nucleic acid would therefore furnish a strong and independent confirmation of the structural formula which Levene and Jacobs have assigned to yeast nucleic acid, and we even venture to suggest that the confirmation would occur at the point of the formula where it is most needed.

The formation of guanylic acid from yeast nucleic acid by the action of the ferments contained in a previously digested extract of pig's pancreas was reported in a preliminary communication by Jones[11], but the results which he described must be regarded as more or less accidental, because

[11] W. Jones (1912), On the formation of Guanylic Acid from Yeast Nucleic Acid. J. Biol. Chem., 12, 31.

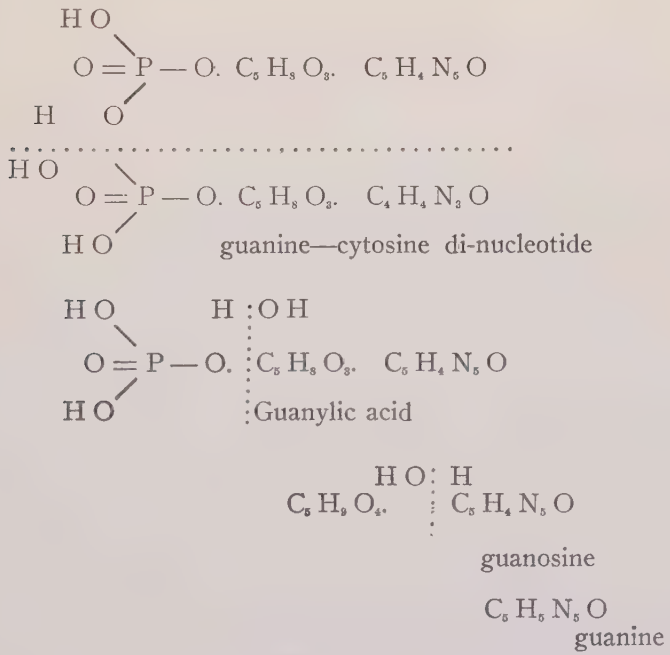
the experiment involves various factors which it is difficult to control, and can be repeated with only occasional success. This will be clear from the following considerations.

When yeast nucleic acid is submitted to the action of an extract of *fresh* pig's pancreas it is first decomposed into two di-nucleotides,



This phase of the decomposition can now be controlled and possession of the two products can be uniformly gained. One of these has been obtained in very pure condition and yields by acid hydrolysis both guanine and cytosine, but neither adenine nor uracil; while the other di-nucleotide yields similarly both adenine and uracil, but neither guanine nor cytosine. Each substance, therefore, contains both a purine and a pyrimidine group, and should lose one-half of its phosphoric acid when submitted to mild hydrolytic agencies. Experiment has shown this to be the case; and these two di-nucleotides are formed from yeast nucleic acid by the action of a ferment present in fresh pig's pancreas (which should obvi-

ously be termed tetra-nuclease) and they represent the *first phase of the enzymatic hydrolysis of yeast nucleic acid.* By continued action of the extract of pig's pancreas other ferments evidently exert their activity and the two di-nucleotides are further decomposed into their constituent mono-nucleotides, one of which is guanylic acid. This constitutes the *second phase of the enzymatic hydrolysis of yeast nucleic acid.* Finally phosphoric acid is removed from the mono-nucleotides in consequence of which the corresponding nucleosides are formed. This is the *third phase of the enzymatic hydrolysis of yeast nucleic acid.* And this progressive decomposition of yeast nucleic acid through di-nucleotides, mono-nucleotides, and nucleosides, with final formation of purine bases, is shown in the following diagram:



But it happens that when the extract of pig's pancreas is allowed to digest at 40° *before adding the yeast nucleic acid* its ferments are gradually destroyed, and in an order inverse to that in which they are exerted upon nucleic acid, so that by properly selecting the duration of this preliminary digestion one should be able to gain possession of a ferment solution which will decompose a nucleic acid to a given point, but no further. In practice, however, this is exceedingly difficult (especially as the duration of digestion is not the only factor which determines the destruction of the ferments), for one will either allow the fresh pancreas to digest for too short a time, in which case its subsequent action on yeast nucleic acid will proceed beyond the decomposition of guanylic acid; or he will allow the fresh pancreas extract to digest for too long a time, and its subsequent action on yeast nucleic acid will not proceed so far as the formation of guanylic acid.

In the great majority of experiments, therefore, guanylic acid will either not be found present among the products of digestion, or will be contaminated with the guanine-cytosine di-nucleotide, since the method of isolation is the same for both substances.

That the physiological factors which bring about the hydrolysis of yeast nucleic acid and its decomposition products are liable to destruction at the body temperature was clearly shown in a study of the action of rabbit's liver extract on guanine. A fresh aqueous extract of this tissue converts guanine into xanthine promptly and completely, so that after digesting with an extract of this tissue for a short time the guanine will have completely disappeared and in its place will be found an equivalent amount of xanthine.

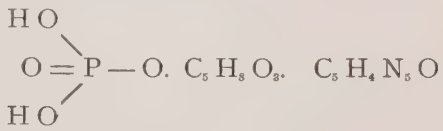
$$C_5 H_3 N_4 O (NH_2) + H_2O = C_5 H_3 N_4 (OH) + NH_3$$

This reaction shows that the ferment "guanase" is present in great activity. If now, the extract of rabbit's liver be allowed to digest at the body temperature its "guanase" becomes destroyed, for guanine added to such a digested extract will remain unaltered, and can be recovered at the end of the digestion, while its deaminization product, xanthine cannot be found.

Not only are these so-called "nuclein" ferments destroyed when their solutions are allowed to remain at the body temperature, but they are curiously sensitive to the "poisonous" effect of the substances which they are expected to decompose. These two matters might lead one to suspect that definite results in this field are difficult or even impossible to obtain. But this is not the case. One sufficiently alert to the possible sources of confusion can obtain results as precise and uniform as are to be secured in any other field of physiological chemistry. In fact, one of the most desirable consequences of the work which is here described was secured by taking advantage of the poisonous action exerted by yeast nucleic acid upon ferments which decompose it. As already stated, a fresh aqueous extract of pig's pancreas can decompose a small initial amount of introduced yeast nucleic acid down to the liberation of purine bases. But if a similar digestion of gland extract be made with a comparatively large initial amount of yeast nucleic acid the enzymatic hydrolysis proceeds only as far as guanylic acid, which can easily be isolated from among the products and completely identified.

In order, therefore, to gain possession of any one of the intermediate products of the enzymatic decomposition of yeast nucleic acid it is necessary either to bring about conditions under which the product in question is formed as an

end product or to find in nature a source of enzyme activity which happens to possess these conditions. For reasons stated above an important intermediate product of yeast nucleic acid and one which throws considerable light upon the structure of the substance is guanylic acid. Two methods have already been described by which this substance with a variable degree of certainty can be obtained, and it will now be shown that guanylic acid is formed as the principal end product of the action of yeast on yeast nucleic acid, so that the one substance can be obtained from the other by a process which involves the exercise of no discrimination by the experimenter and its isolation from the products is effected by a process so simple as scarcely to admit of failure. The guanylic acid thus obtained has been thoroughly identified with the guanylic acid originally found by Bang[12] in the pancreas. It yields sharply the quantities of nitrogen, phosphorus and guanine required for the formula,



liberates its entire phosphoric acid by mild hydrolysis, forms an acid potassium salt, insoluble in alcohol, responds to the pentose color reaction of Tollens,[13] and produces the beautifully crystalline brucine salt which Levene and Jacobs[14] ob-

---

[12] Ivar Bang: loc. cit.

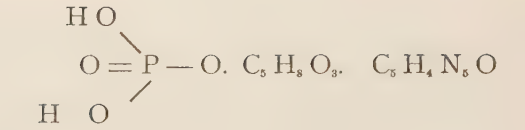
[13] B. Tollens (1896), Ueber den Nachweis der Pentosen mittels der Phloroglucin-Salzsäure-Absatz-Methode. Ber. d. d. chem. Ges., 29, 1202.

[14] P. A. Levene and W. A. Jacobs (1912), On Guanylic Acid. J. Biol. Chem., 47,151.

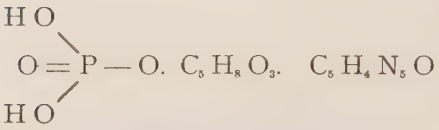
tained with the guanylic acid of the pancreas. In short, there is no observable difference between the substance which we obtained by the enzymatic decomposition of yeast nucleic acid and the pancreatic guanylic acid, whose structure Levene and Jacobs have determined. It cannot be doubted, therefore, that the *molecule of yeast nucleic acid contains a guanylic acid group*.

THE PREPARATION OF GUANYLIC ACID FROM YEAST NUCLEIC ACID.

The experiments referred to in the preceding chapter show that the first decomposition which yeast nucleic acid undergoes in the presence of pancreas extract results in the formation of a di-nucleotide which contains both a guanine and a cytosine group. Arguing from analogy alone it might be supposed that this conduct of yeast nucleic acid is an expression of a weak point in its molecule and that the di-nucleotide will be the first decomposition product formed when yeast nucleic acid undergoes enzymatic hydrolysis under the influence of any hydrolytic ferment whatever. This initial enzymatic hydrolysis, moreover, suggests very clearly that the second stage of the progressive enzymatic decomposition of the nucleic acid will consist in a similar hydrolysis of the di-nucleotide giving rise to guanylic acid, whose structure will necessarily be the same as that of the guanylic acid normally present in animal glands.



guanine—cytosine di-nucleotide.



guanylic acid

Experiment shows that these theoretical considerations represent the facts of the case, for guanylic acid can be found among the products of the action of pancreatic extract on yeast nucleic acid if a fresh extract be used, and the decomposition be arrested at the proper point, either by discontinuing the digestion or by "poisoning" the ferments with an excess of initial yeast nucleic acid. This result may also be reached by the employment of a pancreas extract that has been submitted to a short preliminary digestion at 40°. But in the execution of all these methods special precautions must be observed and particular analytical methods must be applied for the isolation of the product. It happens, however, that yeast contains normally just those ferments that are capable of forming guanylic acid from yeast nucleic acid, and while the decomposition proceeds even to the formation of guanosine and guanylic acid, guanylic acid is present among the end products in much greater amount than either of its two decomposition products, so that its analytical separation can

offer no difficulty. The following method is easily executed and has been found to yield uniform results.

A mixture of 200 grams of Lebedew's commercial yeast powder, 2000 c c of water, and 60 grams of commercial yeast nucleic acid were allowed to digest at 40° in a closed vessel, with enough chloroform to prevent putrefaction. (In addition a control was made with the same materials except that no nucleic acid was added.) From time to time small portions of the clear fluid were withdrawn and tested for nucleic acid by acidification with sulphuric acid. The amount of nucleic acid precipitated in this way was found to diminish continually as the digestion proceeded until the fifth day when the failure of sulphuric acid to produce any precipitate at all with the product showed that the nucleic acid had entirely disappeared. The yellow fluid, while still warm, was siphoned as sharply as possible from the sediment of yeast detritus and allowed to remain in an ice chest over night, when there occurred a copious deposition of flocculent material. On the contrary the fluid from the control gave no precipitate at all when cooled, showing that the substance in question originates from the nucleic acid employed and not from the yeast powder. Moreover the gradually increasing amount of this insoluble substance which is produced as the digestion proceeds in connection with a corresponding gradual disappearance of the initial nucleic acid indicates that we are dealing with a physiological agent of whose activity time is a constant function.

In order to isolate guanylic acid from the digestion products it is only necessary to filter off and manipulate the substance formed by cooling the material, but the yield can be greatly increased by the addition to the fluid before cooling of one-tenth of its volume of absolute alcohol. Under these

conditions the insoluble substance is not only precipitated in greater amount but can be filtered off more easily, and after drying in the air has a tendency to curl at the edges, which makes possible its removal from the filter paper. A further yield of the substance may be obtained by concentrating the filtrate to one-half its volume at 50° under diminished pressure and again cooling in the ice chest after the addition of one-tenth of its volume of absolute alcohol.

The material obtained by the above method is very difficultly soluble in cold water and may be freed from coloring matter and adherent soluble impurities by deposition from a hot aqueous solution as it cools. After several such "pseudo-crystallizations" the product forms perfectly white uniform nodules soluble in about 80 parts of boiling water and 250 parts of water at the room temperature. It is a mixture containing a large proportion of guanylic acid with much smaller quantities of guanosine and guanine. Small quantities of both calcium and magnesium are also present in the material, which though maintaining a fairly constant composition as it is successively "crystallized" does not contain its constituents in a proportion that accords with any assignable formula. The following analyses were made with various specimens dried in a desiccator over sulphuric acid.

I. .2065 g. required 10.50 c c of standard sulphuric acid (1 c c = .0037 g. N)

II. .2078 g. required 10.55 c c of standard sulphuric acid

III. .1367 g. " 6.81 c c " " " "

IV. .1861 g. " 9.39 c c " " " "

V. .6595 g. gave .2660 g. $Mg\,NH_4\,PO_4.\ 6H_2O$

VI. .6530 g. " .2621 g. "

VII. .5081 g. " .2080 g. "

VIII. .4823 g. " .1990 g. "

IX. .6982 g. " .1200 g. $Mg\,NH_4\,PO_4.\ 6H_2O$ (for magnesium)

X. .6982 g. " .0186 g. CaO

FOUND.

	I	II	III	IV	V	VI	VII	VIII	IX	X		
N	18.83	18.78	18.43	18.66								
P					5.26	5.24	5.34	5.39				
Mg									1.6			
Ca										1.9		

.7563 grams of substance were heated for an hour at 100° with 10 c c of 8% sulphuric acid, and the product made strongly alkaline with ammonia. The guanine thus precipitated was filtered off, washed, dried, and weighed. Guanine = .2870. The filtrate from guanine was treated with a solution of silver nitrate in ammonia, and the very small precipitate was decomposed and carefully examined for adenine, but with negative results. This complex mixture contains, therefore, only one purine group, and it is possible to obtain from it only guanine derivatives. By using the following method the substance may be separated into three such derivatives, viz.: guanylic acid, guanosine and guanine.

A solution of the material in warm water is made faintly alkaline with sodium hydroxide, and after filtering off a small amount of precipitated bases is acidified with acetic acid and treated with neutral lead acetate. The guanylic acid is thrown down as a heavy granular lead salt, which, after filtration* and thorough washing with boiling water, is suspended in cold water and decomposed by the passage of sulphuretted hydrogen. The lead sulphide is filtered off, the excess of sulphuretted hydrogen is removed from the cold solution with an air current, and the fluid is concentrated at 50° under diminished pressure. Upon cooling to zero a white granular substance is deposited consisting almost entirely of free guanylic acid, but occasionally contains a small amount of guanine. The latter may be removed by treating the product with warm water, filtering and treating the cold aqueous solution with a large excess of absolute alcohol. Pure guanylic acid is precipitated in perfectly white flocculent form, which is filtered off washed with alcohol and dried in a desiccator over sulphuric acid.

THE PROPERTIES OF GUANYLIC ACID.

The substance described as guanylic acid in the last section when first obtained in its crude form contains traces of guanine. Its phosphorus analysis is for this reason a little low, and its nitrogen correspondingly high.

Various specimens of the crude material, dried to a constant weight at 90° gave the following analytical results.

---

*The filtrate is used for the preparation of guanosine. See below.

I. .5348 g. gave .3272 g. of $Mg\,NH_4\,PO_4.\,6H_2O$

II. .4650 g. gave .2851 g. of $Mg\,NH_4\,PO_4.\,6H_2O$

III. .1471 g. required 7.98 c c of standard acid.
(1 c c = .003695 g. N.)

VI. .1504 g. required 8.24 c c of standard acid

	Theoretical for $H_2PO_4.\,C_5H_8O_3.\,C_5H_4N_5O$	Found I.	II.	III.	IV.
N.	19.28			20.04	20.24
P.	8.55	7.79	7.79		

Various specimens of guanylic acid purified as described and dried to a constant weight at 90° gave the following analytical results:

I. .2783 g. required 14.58 c c of standard acid
(1 c c = .003695 g. N.)

II. .1614 g. required 8.24 c c of standard acid
(1 c c = .003695 g. N.)

III. .2118 g. required 10.88 c c of standard acid
(1 c c = .003695 g. N.)

IV. .5351 g. gave .3431 g. $Mg\,NH_4\,PO_4.\,6H_2O$

V. .5415 g. " .3571 g. "

VI. .5238 g. " .3412 g. "

VII. .4644 g. " .3000 g. "

	Theoretical for $H_2PO_4\,C_5H_8O_3\,C_5H_4N_5O$	I	II	III	IV	V	VI	VII
N	19.28	19.35	18.86	18.98				
P	8.55				8.38	8.37	8.27	8.21

.3800 gram of substance was heated for an hour with 10 c c of 5% sulphuric acid. While still hot the product was made alkaline with ammonia and the precipitated guanine filtered off. It weighed .1499 gram.

	Theoretical for Guanylic Acid	Found
Guanine	41.48	39.45

A drop of the filtrate from guanine failed to give a precipitate with silver nitrate in ammonia. Hence adenine was not present. The bulk of the fluid was then treated with an excess of magnesia mixture and the precipitated magnesium ammonium phosphate was filtered off and weighed.

.3800 gram gave .2370 gram of $Mg\,NH_4\,PO_4.\,6H_2O$

	Theoretical for Guanylic Acid	Found
Total phosphorus	8.55	8.14

This experiment shows that *all* (not half) of the phosphoric acid of this compound is easily liberated by mild hydrolysis. Therefore, the substance does not contain a pyrimidine group.

A portion of guanylic acid dissolved in water was treated with a small amount of a solution of potassium acetate, and the acid potassium salt of guanylic acid was precipitated by the addition of alcohol. The total phosphorus of the substance was determined by fusion, precipitation first with molybdic solution, and then with magnesia mixture.

.6728 gave .4120 g. $Mg NH_4 PO_4 . 6H_2O$

	Theoretical for $C_{10} H_{13} N_5 O_8 PK$	Found
P	7.73	7.99

PREPARATION OF GUANOSINE.

The original filtrate from lead guanylate contains guanosine, which can be easily isolated in the following way: The solution is treated with sulphuretted hydrogen, filtered from lead sulphide and evaporated at 50° under diminished pressure. The addition of neutral lead acetate to this concentrated fluid usually precipitates a small amount of guanylic acid which remains in solution after the former treatment with lead acetate, owing to the greater volume of fluid, but which must be removed at this point; if a finely crystalline specimen of guanosine is to be obtained. The perfectly clear fluid is then treated alternately with small portions of lead acetate and ammonia as long as either reagent produces a precipitate and the precipitated lead compound of guanosine is suspended in hot water and decomposed with sulphuretted hydrogen. After evaporating the filtrate from lead sulphide at 50° under diminished pressure and cooling the concentrated fluid in the ice chest guanosine is deposited in clusters of macroscopic needles, which upon recrystallization from hot water form individual long thin plates with sharp edges.

I. .2176 gram required 12.82 c c of standard sulphuric acid (1 c c = .003695 g. N.)

II. .4374 gram of the substance lost .0470 gram by heating an hour at 105°.

	Theoretical for $C_5H_9O_4$. $C_5H_4N_5O$. $2H_2O$	Found I.	II.
N	21.94	21.76	
$2H_2O$	11.3		10.7

The formation of both guanosine and guanylic acid from yeast nucleic acid of course does not prove that the nucleic acid contains in its structure two guanine groups. It seems more probable that guanylic acid alone is first formed and that part of this is decomposed forming guanosine. The two substances then combine to form the compound which has been described, whose physical properties make possible its easy separation from the other products of digestion, thus furnishing a most convenient method of preparing both guanosine and guanylic acid.

CONCLUSION.

The principal contention of this dissertation is that yeast nucleic acid contains a guanylic acid group, and yields a substance on hydrolysis identical in all respects with the guanylic acid found in the pancreas and other animal glands. This conclusion was placed beyond all doubt by the preparation of guanylic acid from the pancreas and experimenting with the two specimens of the substance side by side, when their properties, even down to unessential details, were found to coincide throughout. While the identity of the two substances is not at all a question of speculation, their natural distributions are almost impossible to explain. The guanylic acid of the pancreas contains and is characterized by a *pentose group* ($C_5H_{10}O_5$), whereas the nucleic acid not only of the pancreas, but of all other animal tissues is equally characterized by its

possession of a *hexose group* ($C_6H_{12}O_6$). It is, therefore, scarcely possible that the guanylic acid of the pancreas is in any way derived from a nuclein precursor in this gland. The easy formation of guanylic acid, however, from yeast nucleic acid, a substance constantly present in animal food, remotely suggests that the guanylic acid of the pancreas is formed from the plant nucleic acid of the food and becomes localized in this tissue. This somewhat speculative reasoning is more to the point if we remember that the production of guanylic acid from yeast nucleic acid now rests upon a firm experimental basis, and that the amount of guanylic acid obtainable from an animal pancreas is in direct proportion to the amount of plant food which it eats. The writer has found this remarkably illustrated in the cases of the pig and the ox, the former of which yields only about one half the amount of guanylic acid that can be obtained from the latter. Moreover, in support of the hypothesis here proposed it may be stated that the guanylic acid of the pancreas which has been frequently described in the literature is indeed not guanylic acid at all, but a compound or mixture of guanylic acid with guanosine, apparently identical with the corresponding compound obtained by the action of yeast on yeast nucleic acid, which has been described in this paper.

BIOGRAPHY

The author of this dissertation was born in Radnor, Pennsylvania, in 1885. She received the degree of A. B. from Bryn Mawr College in 1907 and studied chemistry there for two years as a post graduate. She taught Chemistry and Physiology in the Philadelphia High School for Girls during the year 1910-1911 and was assistant in Chemistry at Barnard College the following year. The year 1912-1913 she spent as a graduate student in Chemistry at the Johns Hopkins University, where she received a scholarship in Chemistry. She has studied during the present year in the Laboratory of Physiological Chemistry of the Johns Hopkins Medical School.